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Effect of Ammonium Salts on Determination of Nicotine

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Ammonium salts interfere in the determination of nicotine by the silicotungstic acid method by retarding the precipitation. Graphs are presented to show the effects of concentrations of nicotine, salt, and reagent and time of digestion on recovery of nicotine. Procedures are proposed to minimize the effect of the salt.

DETERMINATION of nicotine by the gravimetric silicotungstic acid procedure is the official method of the Association of Official Agricultural Chemists (*1*). However, if other alkaloids are present—for example, nornicotine—they are precipitated with nicotine and cause erroneously high results.

Ammonia, another volatile base, has not been considered a possible source of error in this determination, inasmuch as Chapin (*3*) stated that no precipitate was formed when silicotungstic acid was added to a solution containing ammonium chloride. In this laboratory, ammonia was assumed to have no effect until an attempt was made to determine nicotine in a solution that contained nicotine and a fairly high concentration of ammonium salts. When silicotungstic acid was added to this solution, no precipitate formed, either immediately or after the solution had stood overnight. After it had stood for 64 hours in a refrigerator, however, an appreciable precipitate formed, indicating that ammonium salts have a retarding effect on precipitation of nicotine. Consequently, experiments were undertaken to prove or disprove this effect and, if it exists, to determine its magnitude.

METHOD

A stock nicotine solution was prepared by placing a weighed amount of nicotine in a volumetric flask containing sufficient dilute hydrochloric acid to assure an acid solution after it was made to volume with water.

Aliquots containing from 10 to 100 mg. of nicotine were taken from the stock solution and placed in beakers. Four milliliters of hydrochloric acid (1 + 4) were added, and the solutions were diluted to make the volume 100 ml. after the addition of the requisite amount of 12% silicotungstic acid. One milliliter of this reagent was added for each 10 mg. of nicotine. The beakers were placed on a steam bath until the precipitate dissolved, or for 30 minutes, and the solutions were then kept in the refrigerator at about 5° C. for 18 hours, except when the conditions of the experiment required otherwise. The precipitates were collected in tared, ignited Gooch crucibles containing asbestos pads. The precipitates remaining in the beakers were loosened with a rubber policeman, quantitatively transferred to the crucibles, and washed well with dilute hydrochloric acid (1 + 1000). The precipitates were dried under infrared lamps and ignited in a muffle furnace, first at 650° C. for 2 hours and then at 800° C. for 15 minutes. The weight of the residue was multiplied by the theoretical factor 0.1141 to convert the weight of the oxides to the weight of nicotine.

This procedure differs from the A.O.A.C. official method in two respects: The precipitate is digested on a steam bath and then refrigerated overnight instead of merely standing at room temperature overnight, and ignited Gooch crucibles are used for filtering and igniting the precipitate rather than filter paper and platinum crucibles. Use of Gooch crucibles and suction speeds the filtration and eliminates possible errors due to the filter paper.

EXPERIMENTAL

Experiments were conducted in which the following conditions were varied: concentration of ammonium salts, ti-

standing, amount of silicotungstic acid, concentration of nicotine, and temperature.

Experiments were made with both ammonium chloride and ammonium sulfate in concentrations of 0.0 to 18.75%. The time of standing ranged from 18 to 88 hours. The amount of nicotine (about 10 mg.), volume of silicotungstic acid reagent, and temperature during standing (5° C.) were held constant. In each case, 4 ml. of 1 + 4 hydrochloric acid were added, and the final volume was adjusted to 100 ml.

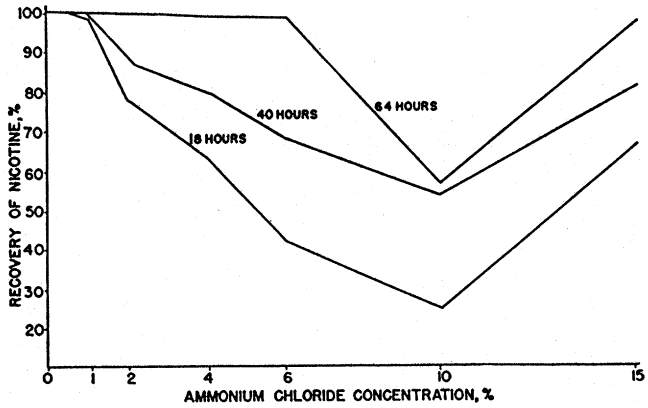

Figure 1. Effect of Concentration of Ammonium Chloride and Time of Standing on Recovery of Nicotine

In each series the amount of nicotine found when no ammonium salt was present was assumed to represent a 100% recovery, and all other recoveries were related to this. Figures 1 and 2 show the effects of time of standing and concentration of ammonium chloride and ammonium sulfate, respectively. A comparison of the two figures shows that the retarding or inhibiting effect of the sulfate salt occurs at a lower concentration, and, except for extremely low concentrations, is not eliminated by long standing. Recoveries for 88 hours (not shown in Figure 2) were essentially the same as for 64 hours.

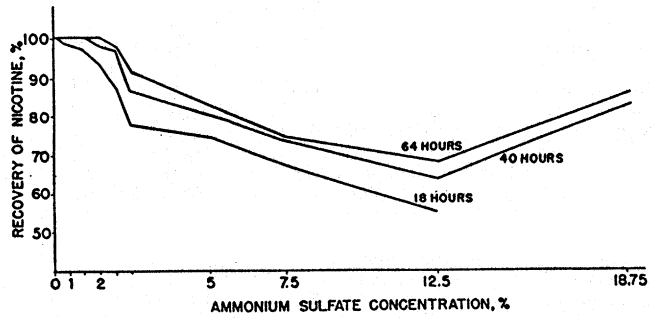

Figure 2. Effect of Concentration of Ammonium Sulfate and Time of Standing on Recovery of Nicotine

Figure 1 shows that as much as 6% ammonium chloride was tolerated if the solution was allowed to stand for 64 hours or longer. When it stood for 18 hours, the period designated in the A.O.A.C. method, 1% of ammonium chloride caused some reduction in recovery of nicotine, whereas 2% lowered it almost one fourth.

Figures 1 and 2 show that concentration of ammonium chloride and ammonium sulfate of 15 and 18.75%, respectively, caused the apparent recovery of nicotine to be greater than that obtained from solutions with 10% ammonium chloride and 12.5% ammonium sulfate. This was probably due to an accompanying coprecipitation of ammonium silicotungstate. That ammonium silicotungstate is precipitated from solutions of high ammonium salt concentration was confirmed by blank analyses on solutions containing 5, 10, and 15% ammonium chloride. Although no precipitate was formed in the 5% solution, there was an appreciable amount in the 10 and 15% solutions, which in terms of nicotine was equivalent to 8.5 and 10.7 mg., respectively. Therefore, solutions containing more than 5 or 6% ammonium chloride should not be analyzed by the silicotungstic acid method.

It was thought that the suppressing or retarding effect of ammonium salts might be avoided by increasing the ratio of silicotungstic acid to nicotine, even though this might involve establishing an empirical factor for converting the weight of the oxides to the weight of nicotine. Consequently, an experiment was conducted in which the amount of silicotungstic acid and the concentration of ammonium salt were varied. The amount of nicotine (10 mg. per 100 ml. of solution) and the time of standing at 5° C. (18 hours) were constant. By increasing the reagent from 1 to 4 ml. per 10 mg. of nicotine, recovery was increased (Figure 3). However, because recoveries at the various salt concentrations did not approach a constant value, a higher ratio of reagent to nicotine was eliminated as a possible remedy for the ammonium salt effect.

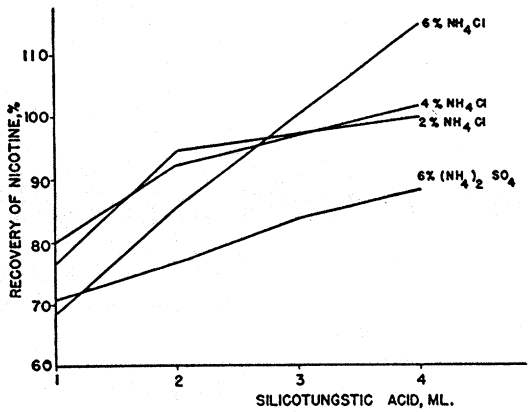

Figure 3. Effect of Concentration of Ammonium Salt and Volume of Reagent on Recovery of Nicotine

The A.O.A.C. method for nicotine analysis recommends that aliquots containing 100 mg. of nicotine be taken for analysis but states that as little as 10 mg. may be used. Because the allowable range of nicotine concentration is from 10 to 100 mg., the effect of ammonium salts was studied at different nicotine concentrations within this range. As shown in Figure 4, better recoveries were obtained at the higher nicotine levels, especially when ammonia was present as the chloride rather than as the sulfate. Complete recovery of nicotine from a 2% ammonium sulfate solution was not obtained even with 100 mg. of nicotine. The curve for ammonium chloride indicates that solutions containing more than 50 mg. of nicotine per 100 ml. can be analyzed satisfactorily with an 18-hour standing period at 5° C. even if they contain the maximum allowable amount (6%) of the salt.

Solutions containing 6% ammonium chloride and less than 50 mg. of nicotine per 100 ml. should not be analyzed by the regula method. There are two possible alternatives: allow the solutions to stand for 64 hours instead of 18, or remove ammonia or prevent its effect on the precipitation. The first alternative gave good recovery (Figure 1), but because of the excessive time required, a search was made for some means of accomplishing the second.

The temperature during standing was about the same (5° C.) for the experiments discussed thus far. Although there was not much hope that changing the temperature would eliminate the effect of the salt, the effect of temperature was studied. One set

of solutions containing about 10 mg. of nicotine per 100 ml. was allowed to stand for 18 hours after precipitation at room temperature, and a second set was kept in a refrigerator at 8° C. The average weight of nicotine recovered was 9.87 mg. for the solutions at room temperature, as compared with 9.90 mg. at 8° C., indicating little or no effect of temperature in the absence of ammonium salts. In the presence of ammonium chloride, however, the corresponding weights—7.52 and 8.68 mg. of nicotine, respectively—showed that the effect of the salt is less pronounced at lower temperatures.

There was a possibility that a readjustment of the pH of the solution might aid in overcoming the effect of the salt. The optimum pH of the solution under normal conditions of analysis is about 1.1, both before and after precipitation of the nicotine. The ammonium sulfate had only slight buffering action, even in concentrations as high as 10%. Therefore the low results with ammonium salts are not due to a change in pH. Because the possibility remained that a pH of 1.1 was not optimum when ammonium salts were present, nicotine solutions containing ammonium sulfate were adjusted to pH 0.6, 1.0, and 2.0 and analyzed for nicotine. None of the recoveries was better than those obtained from similar solutions at pH 1.1. These results indicate that the effect of the salt cannot be suppressed by changing the conditions of the determination.

The possibility that formaldehyde would bind the ammonia, thus removing its effect, was then tested. Although higher nicotine recoveries were obtained from ammonium salt solutions when formaldehyde was added, apparent recoveries greater than 100% were obtained when no salts were present. Consequently, attempts to develop a method based on the use of formaldehyde were abandoned.

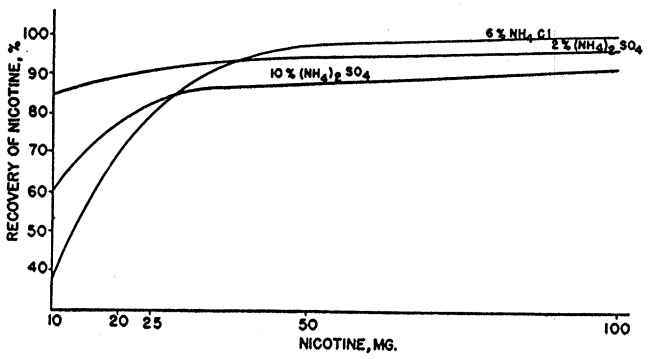

Figure 4. Effect of Amount of Nicotine and Concentration of Ammonium Salt on Recovery of Nicotine

There still remained the possibility of removing the ammonia from nicotine preparations before the distillation of the nicotine or before a redistillation. Markwood (*6*) and Bowen and Barthel (*2*) reported methods for separating nicotine, a tertiary base, from nornicotine, a secondary base, by reaction of the latter with nitrous acid. Because ammonia also reacts with nitrous acid, it was hoped that ammonia could be destroyed in the nicotine solutions without affecting the nicotine; however, such was not the case. At room temperature and below, the reaction of ammonia with nitrous acid or the decomposition of the ammonium nitrite was negligible; at temperatures high enough to decompose the ammonia, the nicotine also reacted with the nitrous acid, with the result that recoveries were lower than those obtained in the presence of ammonium salts.

Treatment of the nicotine-ammonia solution with a hydrogen ion exchange resin was tried to determine whether a preferential adsorption of ammonia or nicotine might be effected, but this has not been successful. Neither a fractional distillation from an ethylene glycol solution according to the method of Libmann-Métayer (*4*) nor the fractional distillation from a phosphate buffer solution as recommended by Neimark (*7*) led to complete recovery of nicotine.

Nicotine solutions containing high ammonium salt concentrations were analyzed by the method described by Markwood (*5*), but apparent nicotine recoveries exceeded 100% when as little as 0.05% ammonium chloride was present.

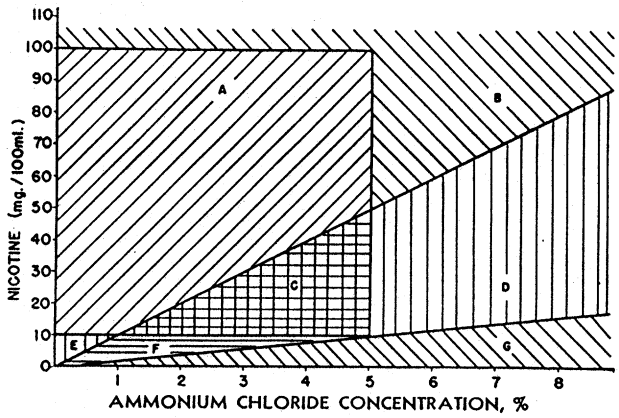

Figure 5. Nicotine Solutions for Which Various Analytical Treatments Are Recommended

The question arose: Was this effect on the precipitation of nicotine silicotungstate peculiar to ammonium salts or was it a general salt effect? Consequently, nicotine solutions containing different amounts of sodium sulfate were analyzed by the silicotungstic acid method. The recoveries paralleled those shown in Figure 2 for ammonium sulfate. Therefore, the low nicotine recoveries from solutions containing ammonium salts are the effect of a salt rather than something peculiar to the ammonium ion. Most of the studies were made with ammonium salts, because they are most likely to be encountered in work with nicotine and because nicotine can be separated from other common salts by steam distillation from an alkaline solution.

SUMMARY

The findings reported here can best be summarized by the schematic diagram, Figure 5. Any solution with nicotine and ammonium chloride concentrations which falls in area A can be analyzed by the Association of Official Agricultural Chemists' procedure, with 18 hours' standing at 5° C. If the nicotine–ammonium salt concentrations fall in area B, the solution must be diluted until area A is reached; the procedure for area A then is used. If a point in area C represents the solution, the analysis should proceed as usual, except that the time of standing at 5° should be at least 64 hours. Solutions represented by points in area D need only be diluted and treated according to the procedure for area C. Solutions whose point falls in area E or F must be concentrated until they are characterized by points in area A or C, respectively, and treated according to the procedure for that area. If characterized by a point in area G, the solution cannot be analyzed by the silicotungstic acid method with accuracy.

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